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Short communication

Hyperproinsulinaemia in impaired glucose tolerance is associated with a delayed insulin response to glucose

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Summary In subjects with impaired glucose tolerance hyperproinsulinaemia has been shown to be predictive for progression to Type II (non-insulin-dependent) diabetes mellitus. These findings are often interpreted as early indicators of an impaired beta-cell function. The aim of our study was to assess the potential determinants of hyperproinsulinaemia in subjects with impaired glucose tolerance. The study group consisted of 110 subjects, 45–74 years of age with mean 2 h plasma glucose concentrations between 8.6 and 11.1 mmol/l following two oral glucose tolerance tests. Subsequently, the hyperglycaemic clamp technique (10 mmol/l, with a priming infusion of 20 % glucose solution, 150 mg/kg) was used to assess the beta-cell function (time needed to reach the insulin peak) and insulin sensitivity (M/I value: glucose metabolised divided by insulin response, 150–180 min). Results showed that the intact-proinsulin:insulin ratio increased with increasing time needed to reach the insulin peak (0.065, 0.079 and 0.101; time needed to reach the insulin peak ≤ 5 min, 5 to 15 min, > 15 min; $p < 0.05$). The split-proinsulin:insulin ratio showed a similar association with the time needed to reach the insulin peak. These associations were independent of age, sex, body mass index and waist:hip ratio. In conclusion, this study shows that relative hyperproinsulinaemia is associated with an impaired beta-cell function in a study group of subjects with impaired glucose tolerance selected after two oral glucose tolerance tests. [Diabetologia (1999) 42: 177–180]

Keywords Impaired glucose tolerance, insulin, proinsulin, hyperglycaemic clamp, beta-cell function, insulin sensitivity.

Plasma concentrations of proinsulin are raised in subjects with Type II (non-insulin-dependent) diabetes mellitus [1]. Proinsulin is normally converted within the beta-cell into split proinsulin and subsequently into insulin and C-peptide [2]. In subjects with impaired glucose tolerance (IGT), the relative (to insulin) proinsulin and absolute proinsulin concentrations are important predictors of progression to Type II diabetes [3, 4]. These findings are often interpreted as early indicators of an impaired beta-cell function but the mechanisms underlying the increase in proinsulin have still not been established. The results of some studies among patients with Type II diabetes have suggested that an inherent beta-cell defect is responsible for raised proinsulin [5, 6], whereas studies with rats [7] and hemipancreatectomised patients [8] have suggested that an increased secretory demand on the beta-cells is responsible. In Type II diabetes, hyperglycaemia per se could contribute to the beta-cell defect. Therefore, studies on subjects with IGT, in which the adverse effects of hyperglycaemia are supposed to be limited, might provide a better insight into the initial underlying cause(s) of (relatively) increased proinsulin concentrations. In this study, 110

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Abbreviations: IGT, Impaired glucose tolerance; OGTT, oral glucose tolerance test; CV, coefficient of variation; IRR, International Reference Reagent.

subjects with IGT were selected, and potential determinants of (relative) proinsulin concentrations, such as beta-cell function, insulin sensitivity, glucose concentration and obesity indices were studied.

Materials and methods

Subjects. Participants were selected from a random sample of 45–74 year-old subjects, taken from the population register of the town of Hoorn in The Netherlands. Of the subjects who received a postal invitation to undergo a blood glucose test (n = 12 093), 55 % visited the study centre. Subjects with a fasting plasma glucose more than 5.5 mmol/l (n = 3147) underwent a complete oral glucose tolerance test (OGTT), which was repeated on another day if the 2-h plasma glucose value exceeded 7.8 mmol/l (n = 554). Subjects with a mean 2-h plasma glucose concentration between 8.6 and 11.1 mmol/l after two OGTTs were invited to participate in the study. This narrow range within the impaired glucose tolerance criterion according to the World Health Organisation (WHO) [9] was chosen to ensure that the participants were definitely at high risk of developing diabetes [4]. Written informed consent was obtained from the participants after the protocol had been approved by the medical ethics committee of the Academic Hospital of the Vrije Universiteit.

Hyperglycaemic clamp study. All 110 participants in the study underwent a hyperglycaemic clamp test [10], in the fasting state and in a supine position, which commenced between 0800 and 0830 hours. Insulin and glucose were measured in venous blood, after arterialization was obtained by placing the hand of the subject in a thermo-regulated box at 45 °C.

Baseline samples were drawn for glucose and insulin measurements, 30 and 15 min before the beginning of the clamp. The clamp started with a priming infusion of a 20 % glucose solution, 150 mg/kg, which was given to rapidly raise the blood glucose concentration to approximately 10 mmol/l. The blood glucose concentration was subsequently measured at 5-min intervals and a variable glucose infusion was adjusted to maintain the plasma glucose concentration at 10 mmol/l for 3 h. Blood samples for insulin measurements were obtained at 2.5 min intervals for the first 10-min, 5-min intervals for the second 10-min and at 10-min intervals for the remaining period of 160 min. Under these stable conditions of constant hyperglycaemia, the amount of glucose infused ($mg \cdot kg^{-1} \cdot min^{-1}$) gives an estimate of the glucose that is metabolised (M). The amount of M divided by the plasma insulin response (insulin in pmol/l) provides an estimate of the tissue sensitivity ($mg \cdot kg^{-1} \cdot min^{-1}$ per $pmol \cdot l^{-1}$) to endogenously secreted insulin in healthy subjects [10], and in subjects with impaired glucose tolerance (unpublished observations). As a variable of beta-cell function an estimate was made of the time needed to reach the insulin peak. The insulin peak was defined as the first highest insulin value after initiation of the clamp, which was followed by an insulin value lower than the 'critical difference' between the two values. The 'critical difference', at a 0.05 level of significance, was defined as 1.96 times 2 times the coefficient of variation for the total within-subject variation [11]. The time needed to reach the insulin peak was used as a variable of the beta-cell function. The first phase insulin response ($pmol \cdot l^{-1} \cdot min^{-1}$), the standard variable of the beta-cell function [10], was not used, since the insulin response during the first 10 min of the clamp was partly absent in this particular study group (Fig. 1).

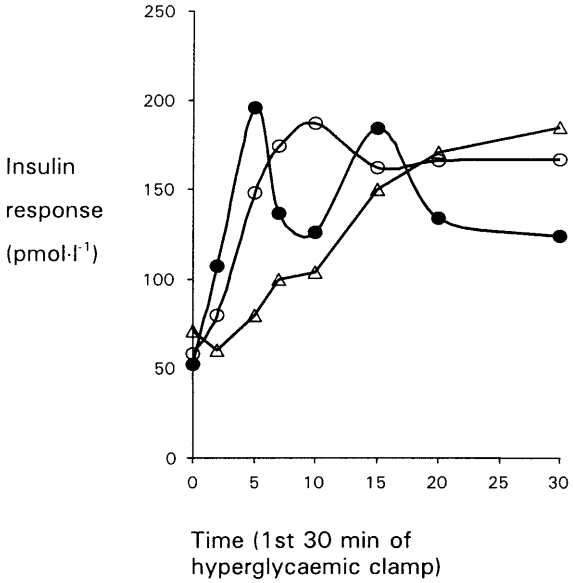

Fig. 1. Insulin response to glucose in subjects with impaired glucose tolerance and a varying beta-cell function. The beta-cell function is expresssed as the time needed to reach the insulin peak: ●, ≤ 5 min; ○, > 5 min and ≤ 15 min; Δ, > 15 min (see text)

Biometry and laboratory analyses. Height, weight, and waist and hip circumference were measured according to standard procedures and used to calculate body mass index (kg/m^2) and waist:hip ratio [12]. Venous whole-blood glucose (mmol/l) was determined by a YSI glucose analyser (YSI, Yellow Springs, Ohio, USA). Specific insulin, intact and total proinsulin were measured in fasting plasma samples drawn before the OGTT (e.g. unheated hand). Insulin was measured by a two-site immunoradiometric assay, using a pair of monoclonal antibodies (Medgenix Diagnostics, Fleurus, Belgium). This assay does not cross-react with proinsulin and 32–33 split proinsulin. The lower limit of detection was 10 pmol/l. The intra-assay coefficient of variation (CV), as determined by replicate analysis of samples, was less than 5 %; the interassay CV was less than 10 % over the concentration range 40–1200 pmol/l. Intact proinsulin was measured by a two-site enzyme immunoassay (K6242, DAKO, Ely, Cambridgeshire, UK), using a pair of monoclonal antibodies. The capture antibody A6 binds to the B-C junction of proinsulin. The detector antibody (3B1) recognises the B10-B16 epitope on the B-chain of proinsulin. This combination of antibodies enables the detection of intact proinsulin along with des 64–65 and 65–66 split proinsulin. These intermediate forms of proinsulin are not normally produced in appreciable quantities, therefore the assay effectively measures only intact proinsulin. The antibodies and a prototype of the assay have been described previously [13]. The assay is calibrated against the first WHO International Reference Reagent (IRR) of proinsulin. The lower limit of detection was 0.5 pmol/l. The intra-assay CV, as determined by replicate analysis of samples over the range 0–90 pmol/l, was less than 4 %. The interassay CV was less than 8 % over the concentration range 4–30 pmol/l. Total proinsulin was measured by a two-site enzyme immunoassay (K6243, DAKO, Ely, Cambridgeshire, UK), using a pair of monoclonal antibodies. The capture antibody (3B1) binds to the B10-B16 epitope on the B-chain of proinsulin. The detector antibody (PEP001) binds somewhere in the C-terminal portion of the proinsulin molecule. This pair of antibod-

ies detects all intermediate forms of proinsulin in addition to the parent molecule. The configuration (3B1 a capture antibody) gives no clinically relevant cross-reaction with either insulin or C-peptide. The antibodies and prototypes of the assay have been described previously [13, 14]. The assay is calibrated against the first WHO IRR of proinsulin. The lower limit of detection was 0.5 pmol/l. The intra-assay CV, as determined by replicate analysis of samples over the concentration range of 0–90 pmol/l, was less than 5%. The interassay CV was less than 7% over the concentration range 8–50 pmol/l. The concentration of split proinsulin (pmol/l) was calculated by subtracting the measured intact proinsulin of the measured total proinsulin. This calculated split proinsulin approximately represents the concentration of 32–33 split proinsulin.

Statistical analysis. Data are presented as medians (33rd and 67th percentile). To show the magnitude of associations between (relative) (pro) insulin concentrations and potential determinants, analysis of covariance and linear regression analysis was applied. Analysis of covariance provides insight into non-linear associations, whereas linear regression analysis provides insight into linear associations. Both methods enable adjustment for the influence of potential confounders. Distributions of the intact and split proinsulin and their ratios were normalised by transformation into their natural logarithm to improve the plots of residual analyses. Results were expressed as geometric means, adjusted for age and sex (analysis of covariance), and as percentage differences in (relative) (pro) insulin (and their 95% confidence intervals) associated with a change of the 33rd to the 67th percentile of the determinant of interest. A zero within the 95% confidence interval implies that no independent statistically significant ($p < 0.05$) association was observed. One subject was regarded as an exception and therefore excluded from the analysis, since the first phase insulin response was twice the value of the second in line. Exclusion of this subject did not substantially affect the results. Analyses were made with the SPSS-PC software package (SPSS, Chicago, Ill., USA).

Results

The clinical and biochemical characteristics of the impaired glucose tolerant study group are shown in Table 1. The median age was 57 years and 50% were women. The study group was characterised by, either an impaired beta-cell function, insulin resistance, or a combination of both defects. Figure 1 depicts the beta-cell function of the study group by the insulin response to the standardised hyperglycaemic stimulus.

In these subjects, the intact and split-proinsulin:insulin ratios were associated with the beta-cell function. The intact-proinsulin:insulin ratio increased with increasing time needed to reach the insulin peak (0.065, 0.079 and 0.101; time needed to reach the insulin peak ≤ 5 min, 5 to 15 min, > 15 min; $p < 0.05$). The split-proinsulin:insulin ratio showed a similar association with the time needed to reach the insulin peak (Table 2). These associations were independent of age and sex. Multiple linear regression analyses confirmed these associations and showed that they were also independent of obesity indices (Table 2). The percentage difference (and 95% CI) in intact and split-proinsulin:insulin ratio associated with the beta-cell function (a difference of 10 min in time needed to reach the insulin peak) was 5% (1 to 9%) and 5% (0 to 9%), respectively. Repeating these analyses, including insulin sensitivity, fasting plasma glucose, 2 h plasma glucose, HbA_{1c}, variables of the liver or kidney function as possible confounders into the regression model, the percentage difference in relative hyperproinsulinaemia associated with the beta-cell function did not shift substantially (data not shown). Thus, the intact and split-proinsulin:insulin ratios were independently associated with a delayed insulin response of the beta-cells to a standardised glucose stimulus.

Table 1. Characteristics of the study group with impaired glucose tolerance

Mean 2 h-plasma glucose concentration between	8.6 and 11.1 mmol/l
Sex (M = men, W = women)	54 M; 55 W
Age (years)	57.0 (52.6–62.0)
Fasting plasma glucose (mmol/l)	6.6 (6.3–6.9)
HbA_{1c} (%)	5.7 (5.4–5.9)
Body mass index (kg/m^2)	27.2 (26.3–29.5)
Waist-to-hip ratio	0.92 (0.90–0.96)
beta-cell function[a] (min)	8 (5–15)
Insulin sensitivity ($100 \times mg \cdot kg^{-1} \cdot min^{-1}$ per $pmol \cdot l^{-1}$)	1.44 (1.01–1.75)
Fasting specific insulin (pmol/l)	69.9 (60.3–88.0)
Fasting intact proinsulin (pmol/l)	5.22 (4.40–7.30)
Fasting split proinsulin (pmol/l)	9.70 (8.00–14.98)
Intact-proinsulin : insulin ratio	0.075 (0.062–0.096)
Split-proinsulin : insulin ratio	0.160 (0.115–0.201)

Values are medians (33rd and 67th percentile) unless otherwise indicated.
[a] The time needed to reach the insulin peak (see text)

Discussion

Our study shows that relative hyperproinsulinaemia is associated with a delayed insulin response to glucose in a homogeneous impaired glucose tolerant study group. The study subjects were at a high risk of developing Type II diabetes, since they were selected on the basis of a mean 2-h plasma glucose concentration between 8.6 and 11.1 mmol/l [4].

The results of some studies suggest that, either an inherent beta-cell defect [5, 6] or an increased secretory demand [7, 8] is responsible for relative hyperproinsulinaemia. The results of our study suggest that an impaired beta-cell function is primaryly responsible for relative hyperproinsulinaemia. Results of an earlier study in the same group showed that an impaired beta-cell function was associated with an increased secretory demand for insulin, represented by a higher concentration of fasting plasma glucose

Table 2. Associations between (relative) (pro) insulin concentrations and beta-cell function in subjects with impaired glucose tolerance

	Beta-cell function, expressed as the time needed to reach the insulin peak			Percentage difference in (relative) (pro) insulin associated with a change of 10 min of the time needed to reach the insulin peak[b]	
(Relative) (pro) insulin	≤ 5 min	> 5 and ≤ 15 min	> 15 min	% difference and 95 %	confidence interval
Specific insulin (pmol/l)	74.5	73.4	65.4	–2	–5 to 1
Intact proinsulin (pmol/l)	4.80	5.74	6.88	3	–1 to 7
Split proinsulin (pmol/l)	10.05	11.08	13.79	3	–2 to 8
Intact-proinsulin : insulin ratio	0.065	0.079	0.101[a]	5	1 to 9
Split-proinsulin : insulin ratio	0.137	0.154	0.203[a]	5	0 to 9

Left hand: values are geometric means adjusted for age and sex, [a] p-value < 0.05 (analysis of covariance)

[b] Right hand: associations are adjusted for age, sex, body mass index and waist-to-hip ratio and calculated with the help of linear regression models (see text)

[15]. In this study, no association was found between relative hyperproinsulinaemia and insulin sensitivity, in contrast to another study [16] in which a negative association was found in 138 people with normal glucose tolerance. The discrepancy between the two studies might be explained by glucose tolerance status or by a small difference in statistical power.

Our results show further that the relative intact and split proinsulinaemia increased in proportion to an impaired beta-cell function, which suggests that the orderly cleavage of proinsulin is maintained. Exhaustion of the beta-cells also seems likely because an association between the proinsulin:insulin ratio and the beta-cell function in hemipancreatectomised patients has been found [8]. An inherent abnormality of the conversion process of proinsulin to insulin [2] cannot, however, be excluded by our study and might be investigated by more detailed experimental studies on the role of intracellular Ca^{2+} metabolism and related signalling pathways for the processing and transport of proinsulin [17]. External factors related to obesity might also affect the proinsulin conversion mechanism, which was suggested by the confounding influence of obesity indices and by a recent study on non-esterified fatty acids [18]. In conclusion, relative hyperproinsulinaemia is associated with the beta-cell function, reflected by a delayed insulin response to a standardised hyperglycaemic stimulus.

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